Exp Appl Acarol (2008) 44:57–60
DOI 10.1007/s10493-008-9128-z

Molecular evidence for a novel *Coxiella* from *Argas monolakensis* (Acari: Argasidae) from Mono Lake, California, USA

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Received: 5 September 2007 / Accepted: 10 January 2008 / Published online: 5 Feburay 2008

Abstract Argasid ticks are vectors of viral and bacterial agents of humans and animals. Recent reports indicate that some ornithophilic argasids harbored rickettsial agents. A Nearctic tick, *Argas monolakensis* Schwan, Corwin, Brown is ornithophilic and has not previously been examined for rickettsial agents. Thirty adult *A. monolakensis* were tested by PCR for DNA from *Rickettsia* or *Coxiella*. Amplicons from a *Coxiella* sp. that were divergent from *Coxiella burnetii* were detected in 16/30 *A. monolakensis.* These molecular isolates were similar but not identical to *C. burnetii*, the *Coxiella* spp. of other ticks, and "*Coxiella cheraxi*" a pathogen of crayfish.

Keywords Tick · *Coxiella* · Intracellular bacteria · *Rickettsia* · *Argas*

Argasid ticks naturally transmit arboviruses and relapsing fever spirochetes (*Borrelia* spp.), but are not generally considered natural vectors of rickettsial agents (Hoogstraal 1985). Recently novel DNA sequences from both *Rickettsia* and *Coxiella* spp. were reported from argasid ticks in North America (Loftis et al. 2005; Reeves et al. 2006a). *Coxiella burnetii* also was reported from wild caught Egyptian *Argas persicus* (Oken) (Loftis et al. 2006). Reeves et al. (2006b) demonstrated laboratory transmission of spotted fever *Rickettsia* from *Carios kelleyi* to (Colley & Kohls) guinea pigs. Few native North American argasids have been tested to determine if they harbor and might transmit rickettsial agents. *Argas monolakensis* Schwan, Corwin, Brown is a bird feeding tick native to Mono Lake, California, USA. Rickettsial agents have never been reported from this species but it is naturally infected with and *Orbivirus* called Mono Lake virus (Schwan et al. 1988). To determine if *A. monolakensis* harbors rickettsial agents, such as *Coxiella* and *Rickettsia* spp., DNA extracts from 30 ticks were tested by PCR for these agents.

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Materials and methods

Argas monolakensis was collected from Little Tahiti Island, Mono Lake, CA, USA on 8 Sep. 2005 by T.G. Schwan and preserved in 95% ethanol. DNA was extracted from 30 individual ticks by freezing them in liquid nitrogen and crushing them with a sterile Teflon pestle. Total DNA was extracted from the pulverized remains with an IsoQuick Nucleic Acid Extraction Kit (ORCA Research Inc., Bothell, Washington) and resuspended in nuclease free water. Teflon pestles were cleaned in 10% sodium hypochlorite for 3 h, rinsed in distilled water, and autoclaved before each use. The extract was screened for the presence of DNA from *Coxiella* and *Rickettsia* by polymerase chain reaction (PCR) using the *mucZ* and 17 kD antigenic gene primers described by Sekeyova et al. (1999) and Carl et al. (1990). PCR conditions consisted of an initial DNA denaturation and hot start at 95°C for 5 min; followed by 40 consecutive cycles of 1 min denaturation at 95°C, primer annealing at 55°C for 30 s, extension at 72°C for 30 s, and a final 10 min extension at 72°C. Positive and negative controls were used in all screens and consisted of genomic DNA extracts of *Coxiella burnetii* and *Rickettsia rickettsii* or distilled water. Control reactions were always the last to be set up in PCR strips and were the last to be loaded into gels. Additional genes were amplified from positive ticks using the *gltA*, 16S rRNA, and IS1111 transposon primers described by Roux et al. (1997), Reeves (2005), and Willems et al. (1994). PCR was performed using a Taq PCR Master Mix Kit (Qiagen, Valencia, California). Each PCR reaction contained 12.5 µl of Taq PCR Master Mix Kit (Qiagen, Valencia, California), 7.5 µl of nuclease free water, 1.25 µl of each primer (1 µmol), and 2.5 µl of DNA extract in water. PCR products were separated by electrophoresis on 2% agarose gels and visualized under ultraviolet light with ethidium bromide. Products were purified with a QIAquick PCR Purification Kit (Qiagen, Valencia, California). Duplicate sequencing reactions were performed with a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, California) using PCR primers, and excess dye was removed with a DyeEx 2.0 column (Qiagen, Valencia, California). Sequences were determined using an ABI 3100 capillary sequencer (Applied Biosystems, Foster City, California), aligned and assembled with Seqmerge (Accelrys, San Diego, California), and compared to sequences in GenBank using the BLAST 2.0 program (NCBI, Bethesda, Maryland).

The mucZ gene sequences from this project, *C. burnetii* and the *Coxiella* of *Carios capensis* were aligned using CLUSTAL W (Supercomputer Laboratory, Kyoto University, Japan) and the phylogenetic relationships of these genes were reconstructed using PHYLIP (Departments of Genome Sciences and Biology, University of Washington) (Fig. 1).

To reduce the possibility of contamination of the DNA extracts or PCR assays, no culturing or cultures of bacterial agents were allowed in the laboratory where the DNA extraction or PCR were performed. All DNA extractions were preformed in a separate area of the laboratory from the PCR setup and gel electrophoresis. All PCR were set up in a containment hood to reduce airflow or contamination. The hood was UV sterilized and wiped clean with ethanol or bleach regularly. None of the DNA fragments detected had previously been in the laboratory where the research was conducted.

Voucher specimens of *A. monolakensis* were deposited in the Georgia Museum of Natural History, University of Georgia, Athens, Georgia. DNA sequences from the *Coxiella* sp.

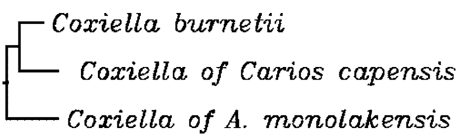

Fig. 1 Reconstruction of the phylogenetic relationships of *Coxiella* spp. based on the mucZ gene

were submitted to GenBank with the following accession numbers: 16S ribosomal RNA gene (EF114360), citrate synthase (*gltA*) (EF114361), and mucZ gene (EF114362).

Results and discussion

Both positive and negative controls produced the expected results. DNA from *Rickettsia* were not detected in any of the samples. DNA sequences from all 16 ticks were identical. A novel *mucZ* sequence from a *Coxiella* sp. was detected in 16 ticks (53%). *Coxiella burnetii* is the sole named species in the genus *Coxiella* (Skerman et al. 1980). However, unnamed *Coxiella* spp. have been detected and partially described from ticks such *Amblyomma americanum* (L.) and *Carios capensis* (Neumann) (Jasinskas et al. 2007; Zhong et al. 2007; Reeves et al. 2006a). The DNA sequences of the agent detected in *A. monolakensis* were not identical to any isolate of *C. burnetii.* Amplification of the IS1111 transposable element of *C. burnetii* from the ticks failed. The 16S rRNA gene sequence (1,128 bp) was 97–98% similar to both *C. burnetii* and the *Coxiella* endosymbionts of argasid and ixodid ticks and 95% similar to "*Coxiella cheraxi*" a pathogen of crayfish. The mucZ gene (733 bp) was 92% similar to *C. burnetii* and 90% similar to the endosymbiont of *C. capensis* and the gltA gene sequence (418 bp) was 91% similar to both *C. burnetii* and the endosymbiont of *C. capensis.* These gene sequences were divergent from *C. burnetii* and indicate that this *Coxiella* is unique. The molecular isolate shared the unique mucZ gene of *Coxiella*, which was similar but not identical to other sequences. The phylogenetic relationship of three *Coxiella* spp. were determined using characters in the mucZ gene (Fig. 1) and *C. burnetii* and the endosymbiont of *C. capensis* were closer related to each other than this new isolate. The lack of an IS1111 transposable element indicates that this was not *C. burnetii*. Alternatively IS1111 transposons could be present but with highly variable sequences. Denison et al. (2007) reported some sequence variability and deletions in individual copies of the IS1111 transposon of *C. burnetii*. Based on the data by Denison et al. (2007) and GenBank sequences the primers described by Willems et al. (1994) should amplify at least one copy of the IS1111 transposon in all *C. burnetii* isolates. Previous researchers have reported *Coxiella* other than *C. burnetii* from both argasid and ixodid ticks (Roshdy 1968; El Shoura 1990; Mediannikov et al. 2003; Lee et al. 2004; Reeves et al. 2006a; Jasinskas et al. 2007). These agents have not been cultured and remain unnamed.

Argas monolakensis harbors a *Coxiella* sp., which is similar to *C. burnetii*, other *Coxiella* reported form ticks, and "*Coxiella cheraxi*" a crayfish pathogen. Future research might indicate that this *Coxiella* can be transmitted to the vertebrate hosts of *A. monolakensis* or that this *Coxiella* is an endosymbiont of the ticks. Additional research is needed to isolate the unnamed *Coxiella* spp. of ticks to determine their antigenic similarity to *C. burnetii* and their pathogenicity to vertebrates.

Acknowledgements I thank T.G. Schwan for giving me access to freshly collected *A. monolakensis* and this project was partially funded by the American Society for Microbiology and the Centers for Disease Control and Prevention. The use of trade names in this document does not constitute an official endorsement or approval of the use of such commercial hardware or software. Do not cite this document for advertisement.

References

Carl M, Tibbs CW, Dobson ME, Paparello S, Dasch GA (1990) Diagnosis of acute typhus infection using the polymerase chain reaction. J Infect Dis 161:791–793

Denison AM, Thompson HA, Massung RF (2007) IS1111 insertion sequences of *Coxiella burnetii*: characterization and use for repetitive element PCR-based differentiation of *Coxiella burnetii* isolates. BMC Microbiol 7:91

El Shoura SM (1990) Ultrastructure and distribution of intracellular rickettsia-like microorganisms in various organs of the laboratory-reared adult tick *Argas* (*Persicargas*) *arboreus* (Ixodoidea: Argaside). Exp Appl Acarol 9:137–143

Hoogstraal H (1985) Argasid and nuttalliellid ticks as parasites and vectors. Adv Parasitol 24:135–238

Jasinskas A, Zhong J, Barbour AG (2007) Highly prevalent *Coxiella* sp. bacterium in the tick vector *Amblyomma americanum*. Appl Environ Microbiol 73:334–336

Lee JH, Park HS, Jang WJ, Koh SE, Park TK, Kang SS, Kim BJ, Kook YH, Park KH, Lee SH (2004). Identification of the *Coxiella* sp. detected from *Haemaphysalis longicornis* ticks in Korea. Microbiol Immunol 48:125–130

Loftis AD, Gill JS, Schriefer ME, Levin ML, Eremeeva ME, Gilchrist MJR, Dasch GA (2005) Detection of *Rickettsia, Borrelia*, and *Bartonella* in *Carios kelleyi* (Acari: Argasidae). J.Med Entomol 42:473–480

Loftis AD, Reeves WK, Szumlas DE, Abbassy MM, Helmy IM, Moriatiry JR, Dasch GA (2006) Rickettsial agents in Egyptian ticks collected from domestic animals. Exp Appl Acarol 40:67–81

Mediannikov O, Ivanov L, Nishikawa M, Saito R, Sidelnikov YN, Zdanovskaya NI, Tarasevich IV, Suzuki H (2003) Molecular evidence of *Coxiella*-like microorganism harbored by *Haemaphysalis conicinnae* in the Russian Far East. Ann NY Acad Sci 990:226–228

Reeves WK (2005) Molecular genetic evidence for a novel bacterial endosymbiont of *Icosta americana* (Diptera: Hippoboscidae). Entomol News 116:263–265

Reeves WK, Loftis AD, Sanders F, Spinks MD, Wills W, Denison AM, Dasch GA (2006a) *Borrelia, Coxiella*, and *Rickettsia* in *Carios capensis* (Acari: Argasidae) from a brown pelican (*Pelecanus occidentalis*) rookery in South Carolina, USA. Exp Appl Acarol 39:321–329

Reeves WK, Streicker DG, Loftis AD, Dasch GA (2006b) Serologic survey of *Eptesicus fuscus* from Georgia, USA for *Rickettsia* and *Borrelia* and laboratory transmission of a *Rickettsia* by bat ticks. J Vector Ecol 31:386–389

Roshdy MA (1968) A rickettsialike (*sic*) microorganism in the tick *Ornithodoros savignyi*; observations on its structure and distribution in the tissues of the tick. J Invert Path 11:155–169

Roux V, Rydkina E, Eremeeva M, Raoult D (1997) Citrate synthase gene comparison, a new tool for phylogenetic analysis, and its application for the rickettsiae. Int J Syst Bacteriol 47:252–61

Schwan TG, Oprandy JJ, Main AJ (1988) Mono Lake virus infecting *Argas* ticks (Acari: Argasidae) associated with California gulls breeding on islands in Mono Lake, California. J Med Entomol 25:381–387

Sekeyova Z, Roux V, Raoult D (1999) Intraspecies diversity of *Coxiella burnetii* as revealed by *com*1 and *mucZ* sequence comparison. FEMS Microbiol Lett 180:61–67

Skerman VBD, McGowan V, Sneath PHA (1980) Approved lists of bacterial names. Int J Syst Bacteriol 30:225–420

Willems H. Thiele D, Frolich-Ritter R, Krauss H (1994) Detection of *Coxiella burnetii* in cow's milk using the polymerase chain reaction (PCR). J Vet Med Ser B 41:580–587

Zhong J, Jasinskas A, Barbour AG (2007) Antibiotic treatment of the tick vector *Amblyomma americanum* reduced reproductive fitness. PLoS ONE 2:e405